Research Note

Thermal Destruction of *Escherichia coli* O157:H7 in Sous-Vide Cooked Ground Beef as Affected by Tea Leaf and Apple Skin Powders[†]

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ABSTRACT

We investigated the heat resistance of a four-strain mixture of *Escherichia coli* O157:H7 in raw ground beef in both the absence and presence of white and green tea powders and an apple skin extract. Inoculated meat was cooked using the sous-vide technique, i.e., the meat was packaged in sterile bags and completely immersed in a circulating water bath at low temperature for a period of time. The bags were cooked for 1 h to an internal temperature of 55, 58, 60, or 62.5°C, and then held from 240 min at 55°C to 10 min at 62.5°C. The surviving bacteria were enumerated by spiral plating onto tryptic soy agar overlaid with sorbitol-MacConkey agar. Inactivation kinetics of the pathogens deviated from first-order kinetics. *D*-values (time, in minutes, required for the bacteria to decrease by 90%) in the control beef ranged from 67.79 min at 55°C to 2.01 min at 62.5°C. *D*-values determined by a logistic model ranged from 36.22 (D_1, the *D*-value of a major population of surviving cells) and 112.79 (D_2, the *D*-value of a minor subpopulation) at 55°C to 1.39 (D_1) and 3.00 (D_2) at 62.5°C. A significant increase ($P < 0.05$) in the sensitivity of the bacteria to heat was observed with the addition of 3% added antimicrobials. *D*-value reductions of 62 to 74% were observed with apple powder and 18 to 58% with tea powders. Thermal death times from this study will assist the retail food industry to design cooking regimes that ensure the safety of beef contaminated with *E. coli* O157:H7.

Escherichia coli O157:H7 is a foodborne, toxin-producing enteropathogen responsible for a hemorrhagic form of colitis, bloody diarrhea, and hemolytic uremic syndrome. Contamination of ground beef with *Escherichia coli* O157: H7 is a potential health hazard and a continuing concern both for consumers and the food service industry *(25)*. Contaminated, undercooked ground beef is one of the primary modes of foodborne transmission of this pathogen *(17)*.

Tea compounds and teas are reported to exhibit antimicrobial activities against foodborne pathogens and against bacterial toxins such as Shiga toxins produced by *E. coli* O157:H7 and other infectious bacteria (reviewed by Friedman *(4)* and Song and Seong *(26)* in 2007). In related studies we found that (i) carvacrol and cinnamaldehyde facilitated thermal destruction of *E. coli* O157:H7 in ground beef *(12)*; (ii) catechins from green tea reduced CFU levels of *Clostridium perfringens* spore germination and outgrowth during cooling of ground beef, chicken, or pork *(11)*; and (iii) computer simulations of activities of seven tea catechins in cell membranes *(24)* paralleled antimicro-

bial activity against *Bacillus cereus* as well as activities against human cancer cells *(5, 8)*.

To extend these findings, the main objective of the present study was to assess the ability of polyphenolic compounds present in white and green tea powders and green tea and apple skin extracts to reduce the heat resistance of a cocktail of *E. coli* O157:H7 in raw ground beef cooked at four temperatures.

MATERIALS AND METHODS

Materials. Whole white tea leaves were obtained from Coffee Bean Direct (Stockton, NJ). To obtain the ground white tea powder, leaves were ground to a powder in a Krups (Medford, MA) stainless steel blade coffee grinder, to pass a 5-μm-pore-size screen. To obtain the white tea water extract, 2 lb of whole tea leaves were steeped in 3.5 liters of boiled water (90°C) for 2.5 h. The infusion was collected by straining the leaves though cheesecloth. The leaves were reextracted with 2 liters of boiled water. The combined liquids were centrifuged at $2,500 \times g$ for 20 min and then filtered though Whatman no. 4 filter paper (Kent, UK). The resulting liquid was then frozen in trays and lyophilized in a bulk freeze-dryer, for a yield of 15%. The dried product was passed through a 0.5-mm-pore-size screen to obtain a fine powder. The green tea powder was Chromadex BRM, standardized tea leaves (catalog no. ASB-00030330) obtained from Chromadex, Inc., Santa Ana, CA. The green tea extract was green tea poly-

phenols (catalog no. G6817) obtained from LKT Laboratories, Inc., St. Paul, MN. The Apple E was an apple skin extract containing 82% polyphenols obtained as Apple Poly from Apple Poly LLC, Littleton, CO.

Bacterial cultures. The four strains of *E. coli* O157:H7 used in this study were 45753-35, 933, A9218-C1, and ent C9490 (Jack-in-the-Box). Strains 45753-35 and 933 are meat and kidney isolates, respectively, and were originally obtained from the Food Safety and Inspection Service, U.S. Department of Agriculture (USDA), Beltsville, MD. Strains A9218-C1 and ent C9490 are clinical isolates and were originally obtained from the Center for Disease Control, Atlanta, GA. The strains were preserved at −70°C in vials containing brain heart infusion broth (Difco, Becton Dickinson, Sparks, MD) supplemented with 10% (vol/vol) glycerol (Sigma, St. Louis, MO) and were obtained from the Microbial Food Safety Research Unit laboratory culture collection. Test cultures were prepared and maintained as described previously *(12)*. The population density in each cell suspension was determined by spiral plating (model D, Spiral Biotech, Bethesda, MD) appropriate dilutions (in 0.1 % peptone water), in duplicate, onto tryptic soy agar (TSA; Difco) plates. Equal volumes (2 ml) of each of the four cultures of *E. coli* O157:H7 were combined in a sterile test tube to obtain a cocktail (9 log CFU/ml) for the inoculation of ground beef.

Ground meat: sample preparation and inoculation. Raw, 75% lean ground beef, obtained from a local supermarket, was separated into 300-g batches for the different treatments and mixed thoroughly with tea or tea extract (3%, vol/wt) for 2 min in a KitchenAid mixer (model K45SS, KitchenAid Inc., Greenville, OH). Separate 75-g batches of the mixed ground beef were prepared, vacuum packaged, and stored frozen (−5°C) until use (approximately 60 days). Thereafter, the *E. coli* O157:H7 cocktail inoculum was added (0.15 ml) to 75 g of thawed (over a period of 24 h at 4°C) beef, to obtain a final concentration of ∼8 log CFU/g. Each bag of meat was then blended with a Stomacher 400 Lab-blender (Tekmar, Cincinnati, OH) for 5 min, to ensure even distribution of the organisms in the respective menstruums. Duplicate 5-g meat samples were then weighed aseptically into sterile filtered stomacher bags (30 by 19 cm; Spiral Biotech, Boston, MA). Bags containing meat samples inoculated with 0.1 ml of 0.1% (wt/vol) sterile peptone water with no bacterial cells were used as negative controls. Thereafter, the bags were compressed into a thin layer (∼1 to 2 mm thick) by being pressed against a flat surface, excluding most of the air, and then vacuum sealed at 17 mbar using a Multivac (model A300/16, Multivac, Kansas City, MO) packaging machine.

Sous-vide (cook-in-bag) cooking, thermal inactivation, and bacterial enumeration. To simulate the conditions that occur in the retail food industry and institutional food service settings, the vacuum-packaged bags containing the meat samples were cooked in a water bath as described previously *(14)*. The temperature of the water bath was programmed to increase in a linear fashion to a target temperature of 55, 57.5, 60, or 62.5°C in 1 h. Bags for each replicate were then removed at predetermined time intervals; the sampling frequency was based on the heating temperature, e.g., 15 min at 55°C and 0.5 to 1 min at 62.5°C. A 240-min total heating time at 55°C to 10 min at 62.5°C was used for all experiments. After removal, bags were immediately plunged into an ice-water bath until analyses were performed (within 30 min). Negative controls included bags containing uninoculated meat. Surviving bacteria were enumerated by surface plating appropriate dilutions in duplicate on TSA by using a spiral plater (model D,

Spiral Biotech) by the procedure described previously *(12)*. The TSA plates were overlaid with 10 ml of sorbitol-MacConkey agar (pretempered to 47°C; Oxoid, Lenexa, KS) after 120 min of resuscitation at room temperature to allow recovery of heat-damaged cells *(13)*. After overlaying, the plates were allowed to solidify for 30 min. All plates were incubated at 30°C for at least 48 h prior to counting colonies. Isolates from plates were randomly selected and subjected to serological confirmation as *E. coli* O157:H7 serotype (RIM, *E. coli* O157:H7 Latex Test; Remel, Lenexa, KS). For each replicate experiment, the average CFU per gram value of four platings of each sampling point was used to determine the *D*-values.

Calculation of *D*- and *z*-values. The *D*-values (time to inactivate 90% of the population) were calculated from the straight-line portion of the survival curves by plotting the log of survival counts versus their corresponding heating times, using Excel software (Microsoft Corporation, Seattle, WA). Only survival curves with more than five values in the straight-line portion, with a correlation coefficient (r^2) of >0.90, and descending more than 5 log cycles were used. Also, regression lines were fitted to the experimental data points that contributed to tailing or shouldering by a survival equation (logistic model) using a curve-fitting program (DataFit for Windows, version 7.1, Oakdale Engineering, Oakdale, PA). Two *D*-values (D_1 and D_2) and lag periods (time period before any cell death was observed) were calculated. The *z*-values (change in heating to change the *D*-values by 90%) were estimated by computing the linear regression *(22)* of mean log *D*-values versus their corresponding heating temperatures, using Excel software. The *z*-value was estimated by taking the absolute value of the inverse slope.

Statistical analysis. Analysis of variance using SAS *(23)* was used to calculate significant differences among the treatments, the Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means *(18)*, and the coefficient of multiple determination (square of the correlation coefficient) was used to estimate the proportion of variability of the response of the regression of the logistic model of the kinetics of inactivation of the bacteria.

RESULTS AND DISCUSSION

Composition of test substances. The commercial apple polyphenol product contains 82% polyphenols derived from skins of immature apples through water and ethanol extraction. Primary polyphenols in the extract include procyanidins, catechin, epicatechins, and chlorogenic acid *(16)*. We do not know the distribution of individual phenolic compounds in the apple extract, only the total amount. The composition of the low (∼20%)–catechin green tea powder and high (∼80%)–catechin green tea extract is given in previous publications *(6, 7, 11)*. White teas used in the present study were not analyzed for catechin content. The catechin content of another white tea brand is reported elsewhere *(5, 6)*.

Increased heat sensitivity of *E. coli* O157:H7 in ground beef. The log values of surviving *E. coli* O157:H7 cells per gram of beef were plotted against exposure time at each test temperature. For inoculated control beef heated at 55°C, the *E. coli* O157:H7 count decreased from 7.09 to 6.63 log CFU/g after 60 min of heating. The corresponding value at 57.5°C was 3.26 log (from 7.32 to 4.06 log

TABLE 1. *Heat resistance results, using linear regression, for* E. coli *O157:H7 cocktail in ground beef with 3% tea, tea extracts, or apple polyphenols*

Temp (°C)	Additive	Mean *D*-value ± SD (% of control)[a]	$(r^2)^b$
55	Control	67.79 ± 5.48 (100)	0.97
	3% White tea, ground powder	43.84 ± 2.23 (64.7)	0.96
	3% White tea, water extract	58.92 ± 2.27 (81.9)	0.98
	3% Green tea powder	48.27 ± 1.97 (77.2)	0.96
	3% Green tea extract	40.15 ± 3.45 (59.2)	0.91
	3% Apple E (apple polyphenols)	17.63 ± 5.74 (26.0)	0.93
57.5	Control	21.85 ± 3.84 (100)	0.97
	3% White tea, ground powder	13.34 ± 0.25 (61.0)	0.97
	3% White tea, water extract	15.06 ± 0.53 (68.9)	0.96
	3% Green tea powder	15.11 ± 0.43 (69.2)	0.98
	3% Green tea extract	12.48 ± 0.11 (54.1)	0.96
	3% Apple E (apple polyphenols)	7.23 ± 0.34 (33.1)	0.96
60	Control	5.39 ± 0.17 (100)	
	3% White tea, ground powder	3.67 ± 0.36 (68.1)	0.96
	3% White tea, water extract	2.98 ± 0.02 (55.3)	0.97
	3% Green tea powder	2.67 ± 0.40 (49.5)	0.97
	3% Green tea extract	1.42 ± 0.10 (26.3)	0.97
	3% Apple E (apple polyphenols)	2.06 ± 0.07 (38.3)	0.97
62.5	Control	2.01 ± 0.43 (100)	0.98
	3% White tea, ground powder	1.10 ± 0.20 (54.7)	0.96
	3% White tea, water extract	1.08 ± 0.04 (53.7)	0.97
	3% Green tea powder	1.27 ± 0.05 (63.2)	0.99
	3% Green tea extract	0.85 ± 0.09 (42.3)	0.96
	3% Apple E (apple polyphenols)	0.60 ± 0.08 (29.8)	0.98

[a] *D*-values (in minutes) were obtained by linear regression and are the results of two replicate experiments, each performed in duplicate.
[b] Correlation coefficient.

CFU/g). Heating beef supplemented with the five test substances at 55°C for 60 min resulted in the following reductions in log CFU: white tea powder, 0.74; white tea extract, 0.75; green tea powder, 1.50; green tea extract, 2.17; and apple polyphenols, 2.92. The corresponding reductions at 57.5°C were white tea powder, 4.28; white tea extract, 3.50; and green tea powder, 3.83. Samples for green tea extract and apple polyphenols were not analyzed. At higher temperatures of 60 and 62.5°C, similar increased rates of destruction of *E. coli* O157:H7 were observed in beef supplemented with additives. Thus, the heat resistance of *E. coli* O157:H7 in beef appears to be temperature dependent.

Survivor curves exhibited an initial lag period (shoulder) before any death occurred. The observed "shoulder effect" may result from poor heat transfer throughout the beef matrix *(10)*.

The *D*-values of *E. coli* O157:H7 in beef at 55, 57.5, 60, and 62.5°C are presented in Tables 1 and 2. The *D*-values calculated by linear regression of the data obtained with control beef ranged from 67.79 min at 55°C to 2.01 min at 62.5°C (Table 1). Addition of 3% test substances to beef rendered the pathogen more sensitive to the lethal effect of heat at all temperatures, as indicated by lower recovery of heated *E. coli* O157:H7 cells. Table 1 shows that compared with the control values, the reduction in *D*-values (in percentages) at 55°C ranged as follows: white tea extract, 13.0; green tea powder, 28.8; white tea powder, 35.3; green tea extract, 40.8; and apple polyphenols, 74.0. Similar increas-

es in the sensitivity of the pathogen to heat were observed at higher temperatures in beef supplemented with 3% test substances. Regression curves calculated for the four temperatures (55, 57.5, 60, and 62.5°C) correlated with r^2 values of >0.91. These data show that the apple skin extract was the most active formulation in beef, rendering significantly increased ($P < 0.05$) heat sensitivity of *E. coli* O157: H7.

Differences between *D*-values obtained in this and other studies *(1, 3, 15)* may be attributed to differences in the strains used, growth phase of the cells, the pH or fat content of meat, and different methodologies used to quantify the heat destruction of bacteria and/or recovery medium used to enumerate the survivors.

To calculate the time required to achieve a specific lethality at a specific temperature for *E. coli* O157:H7 in beef, lag periods must be added to the observed *D*-values. *D*-values calculated from only the linear portion of the survivor curves, which ignores shoulders or lag periods, could lead to an underestimation of the times and temperatures needed to achieve a target reduction in cell numbers. Therefore, the survivor curves were also fitted using the logistic function that allows for the presence of a lag period and tailing. These curves provide two *D*-values: one for a major population (D_1) and a second for a subpopulation (D_2) of surviving cells.

Trends in increased sensitivity of *E. coli* O157:H7 cells in beef with added test substances were also apparent from

TABLE 2. *Heat resistance results, obtained by the logistic model, for* E. coli *O157:H7 cocktail in ground beef with 3% tea, tea extracts, or apple polyphenols[a]*

Temp (°C)	Additive	D_1	D_2	T_L (min)	$D_1 + T_L$ (min)	$D_2 + T_L$	CMD (R^2)
55	Control	36.22 ± 3.46	112.79 ± 3.13	58.10 ± 10.53	94.31 ± 7.07	170.89 ± 13.65	0.99
	3% White tea, ground powder	51.30 ± 8.78	66.05 ± 23.60	18.59 ± 13.83	69.89 ± 5.06	84.64 ± 37.43	0.99
	3% White tea, water extract	57.39 ± 12.84	60.90 ± 19.47	7.03 ± 9.93	64.42 ± 2.91	67.93 ± 9.54	0.99
	3% Green tea powder	37.08 ± 8.24	72.90 ± 13.48	5.18 ± 7.32	42.26 ± 0.93	78.07 ± 20.80	0.98
	3% Green tea extract	27.04 ± 11.12	89.96 ± 7.19	0.00 ± 0.00	27.04 ± 11.12	89.96 ± 7.19	0.97
	3% Apple E (apple polyphenols)	10.05 ± 4.91	48.16 ± 6.50	34.45 ± 3.22	44.49 ± 1.70	82.60 ± 3.28	0.99
57.5	Control	21.13 ± 9.55	29.68 ± 28.43	7.81 ± 1.96	28.94 ± 11.50	37.49 ± 26.47	0.99
	3% White tea, ground powder	12.14 ± 0.91	15.33 ± 0.00	11.58 ± 4.09	23.72 ± 3.18	26.91 ± 4.09	0.99
	3% White tea, water extract	9.06 ± 1.97	14.43 ± 1.27	26.10 ± 1.76	35.16 ± 0.21	40.53 ± 0.49	0.99
	3% Green tea powder	5.67 ± 0.78	16.43 ± 0.00	6.76 ± 1.38	12.42 ± 0.59	23.19 ± 1.38	0.99
	3% Green tea extract	6.95 ± 3.19	17.69 ± 0.00	0.69 ± 0.97	7.63 ± 2.22	18.38 ± 0.97	0.98
	3% Apple E (apple polyphenols)	5.97 ± 2.06	37.99 ± 38.95	7.25 ± 2.21	13.22 ± 0.15	45.24 ± 41.15	0.99
60	Control	5.68 ± 0.10	9.72 ± 2.52	0.00 ± 0.00	5.68 ± 0.10	9.72 ± 2.52	0.99
	3% White tea, ground powder	1.41 ± 0.24	5.13 ± 1.55	1.48 ± 0.03	2.89 ± 0.27	6.61 ± 1.52	0.99
	3% White tea, water extract	2.72 ± 0.07	1.97 ± 0.23	2.45 ± 0.31	5.17 ± 0.38	4.42 ± 0.08	0.99
	3% Green tea powder	0.56 ± 0.46	2.80 ± 0.49	3.84 ± 0.24	4.40 ± 0.22	6.64 ± 0.25	0.99
	3% Green tea extract	1.11 ± 0.25	2.55 ± 0.23	0.58 ± 0.13	1.69 ± 0.12	3.13 ± 0.35	0.98
	3% Apple E (apple polyphenols)	2.00 ± 0.23	2.25 ± 0.28	1.26 ± 0.29	3.26 ± 0.06	3.50 ± 0.01	0.98
62.5	Control	1.39 ± 0.64	3.00 ± 1.74	0.71 ± 1.00	2.09 ± 0.35	3.82 ± 0.74	0.99
	3% White tea, ground powder	1.12 ± 0.06	0.64 ± 0.91	0.32 ± 0.45	1.44 ± 0.39	0.96 ± 1.36	0.98
	3% White tea, water extract	1.03 ± 0.24	1.06 ± 1.39	0.70 ± 0.99	1.73 ± 0.75	1.76 ± 0.40	0.98
	3% Green tea powder	0.94 ± 0.32	1.92 ± 0.75	0.30 ± 0.42	1.23 ± 0.10	2.22 ± 0.33	0.99
	3% Green tea extract	0.48 ± 0.07	1.04 ± 0.78	0.67 ± 0.25	1.15 ± 0.18	1.71 ± 1.03	0.99
	3% Apple E (apple polyphenols)	0.60 ± 0.06	1.26 ± 0.35	0.06 ± 0.08	0.66 ± 0.14	1.32 ± 0.27	0.99

[a] *D*-values (in minutes) are the means of two replicate experiments, each performed in duplicate and expressed as means ± SD. D_1, *D*-value of a major population; D_2, *D*-value of subpopulation; T_L, shoulder or lag period, R^2, coefficient of multiple determination.

the D-values obtained by using a logistic model (Table 2). Calculated D-values (in minutes) in control beef ranged from 36.22 (D_1) and 112.79 (D_2) at 55°C to 1.39 (D_1) and 3.00 (D_2) at 62.5°C (Table 2). In the present study, lag periods were added to the observed D-values. Significantly increased ($P < 0.05$) sensitivity of the bacteria to heat at all temperatures was observed with the addition of 3% added antimicrobials. For example, D-values at 55°C significantly ($P < 0.5$) decreased in beef to 10.05 (D_1) and 48.16 (D_2) for the apple extract. General decreases in lag periods were observed in beef at all temperatures tested in the presence of additives. As would be expected, shorter lag periods appeared to increase the sensitivity of the cells to heat, resulting in lower D-values.

To calculate z-values (thermal death time values), we plotted thermal death time curves using D-values obtained following heating the *E. coli* O157:H7 cells in beef, with or without additives (the plots are not shown). The z-values (in degrees Celsius) in meat ranged from 4.23 (beef with no additives) to 5.06 (beef with additives; calculated from D-values obtained by a linear regression). The plotted data indicate that uniform heat lethality kinetics appears to govern the inactivation of the pathogens. This conclusion is based on the similarity of slopes of the z-value lines for *E. coli* O157:H7 cells in beef from experiments with or without additives.

The calculated z-values using D_1-values (in minutes) determined by a logistic model ranged from 5.19 (beef with no additives) to 6.03 (beef with additives). A possible explanation for the changes in z-values for beef with additives may be that the bacterial cells exhibit varying degrees of lag phase in beef with additives. Because cells heated at different temperatures exhibited varying lag periods, to calculate z-values we added the observed lag periods to the D-values. The z-values in meat, obtained using D_1-values calculated by a logistic model with added lag periods, ranged from 4.41 (beef with no additives) to 5.24 (beef with 3% green tea extract). By using D_2-values calculated by a logistic model, the z-values ranged from 4.80 (beef with no additives) to 4.18 (beef with 3% apple polyphenols).

Our data show that the z-values in meat, with or without additives, were similar to those with lag periods added to the observed D_2-values; the values with added lag periods ranged from 4.51 (beef with no additives) to 3.85 (beef with 3% apple polyphenols). These findings suggest that for cocktails of *E. coli* O157:H7 strains evaluated in beef containing antimicrobials, higher changes in temperature and lower changes in D_2-value are required to cause 90% reduction in the D_1-value than in analogous experiments without the additives. These observations suggest that it would not be advisable to determine z-values under one set of food formulations *(12, 14, 15)* and apply it to another set.

Relative activities of tea and apple preparations. The data in Tables 1 and 2 show that (i) the four white and green tea preparations induced similar reductions in D-values; and (ii) with the exception of the green tea extract at 60°C, the apple polyphenol preparation was most active

against the pathogen. It reduced the D-values by about two-thirds, compared with the control. The cited observations suggest that apple skin powder is a good candidate for further evaluation as a natural antimicrobial in food.

In summary, the results of the previous study with *C. perfringens (11)* and the present study with *E. coli* O157:H7 indicate that tea and apple extracts containing phenolic compounds can significantly reduce the temperature required to kill foodborne organisms. The data presented in Tables 1 and 2 can be used to predict the time required at specified temperatures to achieve a specified reduction of *E. coli* O157:H7 when heated in beef supplemented with tea and apple powders. Contaminated sous-vide processed ground beef should be heated to an internal temperature of 57.5°C for at least 87.4 min to achieve 4-D reduction. The heating times at 57.5°C to achieve the same effect can be reduced to 28.92 min in beef with 3% apple extract added. Thermal death time values from this study should assist the retail food industry and food processors to design cooking regimes that require less energy to eliminate *E. coli* O157:H7 in sous-vide cooked 75% lean beef. These results complement and extend related observations on the control *C. perfringens* spores by green tea leaf extracts during cooling of cooked ground beef, chicken, and pork *(11)*. In addition, our finding that D-values obtained by linear regression are similar to those obtained by the logistic model should stimulate interest in finding out whether this is generally the case with other antimicrobials.

It is also relevant that other investigators reported that antioxidative tea extracts increased the shelf life of beef patties *(2, 21, 27)*, inhibited lipid oxidation in raw beef to a greater extent than did vitamin C *(19)*, improved the functionality (lipid oxidation, trapping of free radicals, and sensory properties) *(9)* and degradation of vitamin E in cooked pork patties *(20)*, and inhibited the formation of heterocyclic amine mutagens during cooking of meat *(28)*. These observations and the results of the present study imply that tea polyphenolic compounds have the potential to impart multiple beneficial functional, nutritional, and antimicrobial properties to meat.

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